

This listing of claims will replace all prior versions and listings of claims in the application.

1 - 25. (Canceled)

26. (Currently amended) A method of determining the presence and sequence of at least one target polynucleotide in a sample comprising:

combining nucleic acid from the sample with at least one set of reaction compositions comprising a first reaction composition and second reaction composition, both specific for the at least one target polynucleotide, wherein the first reaction composition comprises amplification primers specific to the at least one target polynucleotide and lacks a fluorescent indicator, and the second reaction composition comprises a fluorescent indicator and amplification primers specific to the at least one target polynucleotide, and wherein the first reaction composition and the second reaction composition are separate reaction compositions;

amplifying the at least one target polynucleotide present in the reaction compositions using the amplification primers to obtain at least one amplification product;

irradiating the at least one amplification product of the second reaction composition such that the fluorescent indicator produces a fluorescent signal, wherein the intensity of the fluorescent signal is related to the quantity of the at least one amplification product;

monitoring the amplifying of the second reaction composition by detecting the fluorescent signal from the fluorescent indicator;

determining whether the at least one amplification product is present in both the first reaction composition and the second reaction composition from the intensity of signal from the fluorescent indicator in the second reaction composition; and

determining the sequence of the at least one amplification product of the first reaction composition if the at least one amplification product is present in the first reaction composition.

27. (Original) The method of claim 26, wherein the determining of the sequence comprises:

performing a sequencing reaction on the at least one amplification product to obtain a sequencing product; and

placing the product of the sequencing reaction into a sequencing apparatus to determine a sequence of the at least one amplification product.

28. (Original) The method of claim 27, wherein the fluorescent signal is detected using a device.

29. (Original) The method of claim 28, wherein the amplifying, irradiating, and monitoring comprise use of a thermal cycler, a device that irradiates the at least one amplification product, a device that detects resulting fluorescence during each cycle, and a device that displays the increase in fluorescence by cycle number.

30. (Original) The method of claim 29, wherein the thermal cycler, the device that irradiates, the device that detects, and the device that displays are all components of a single device.

31. (Original) The method of claim 28, wherein nucleic acid from the sample is combined with at least two different sets of separate reaction compositions comprising the first reaction composition and the second reaction composition, wherein each set of separate reaction compositions comprises amplification primers specific for a different target polynucleotide, and wherein the amplifying results in different amplification products if the different target polynucleotides are present in the sample.

32. (Original) The method of claim 29, wherein the amount of amplification product and number of cycles are used to determine the amount of target polynucleotide present in the sample prior to the amplification.

33. (Original) The method of claim 28, wherein the monitoring occurs after the amplifying is complete.

34. (Original) The method of claim 29, wherein the monitoring occurs during two or more cycles during the amplifying.

35. (Previously presented) The method of claim 28, wherein the nucleic acid from the sample is derived from at least one biological source selected from a virus, a prokaryote, a protist, a plant, a fungus, and an animal.

36. (Previously presented) The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of a pathogen, wherein the pathogen is at least one pathogen selected from a virus, a prokaryote, and a eukaryote.

37. (Previously presented) The method of claim 36, wherein the pathogen is at least one pathogen selected from HIV, specific *E. coli* strains, *Salmonella species*, and *Haemophilus species*.

38. (Previously presented) The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of at least one of a genetic disease and/or a genetic predisposition to a disease.

39. (Original) The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of a specific allele.

40. (Original) The method of claim 39, wherein the presence of the specific allele indicates serotype.

41. (Previously presented) The method of claim 39, wherein the presence of one or more specific alleles indicates one or more cell surface proteins which determine at least one HLA type.

42. (Previously presented) The method of claim 41, wherein the at least one HLA type comprises at least one HLA type selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-DOB, HLA-DMA, and HLA-DMB.

43. (Previously presented) The method of claim 28, wherein the presence of a given target polynucleotide indicates the presence of at least one polynucleotide selected from TAP1, TAP2, and MICA.

44. (Original) The method of claim 28, wherein the fluorescent indicator is a nucleic acid binding molecule.

45. (Original) The method of claim 44, wherein the fluorescent indicator is an intercalating dye.

46. (Original) The method of claim 44, wherein the fluorescent indicator is a minor groove binding molecule.

47. (Original) The method of claim 44, wherein the fluorescent indicator is a molecular beacon.

48. (Previously presented) The method of claim 28, wherein the fluorescent indicator is at least one fluorescent indicator selected from SYBR® Green I; thiazole orange; ethidium bromide; pico green; acridine orange; quinolinium 4-[(3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; quinolinium 4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]-1-[3-(trimethylammonio) propyl]-diiodide; and chromomycin A3.

49. (Original) The method of claim 28, wherein the fluorescent indicator is a 5'-nuclease fluorescent indicator.

50. (Previously presented) The method of claim 28, wherein the sample is at least one sample selected from whole blood, a tissue biopsy, bone marrow, semen, sputum, urine, amniotic fluid, sperm, hair, skin, and cultured cells.

51-68. (Canceled)